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Key Points:
- We examined environmental factors associated with algal blooms, *Vibrio vulnificus*, and *V. parahaemolyticus* in coastal stormwater ponds
- *Vibrio* incidences were positively associated with temperature, followed blooms during warmer months, but not correlated with chlorophyll
- Algal blooms may augment *Vibrio* in systems considered here under future environmental conditions, posing public health concerns

Supporting Information:
- Supporting Information S1

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Temporal and Environmental Factors Driving *Vibrio Vulnificus* and *V. Parahaemolyticus* Populations and Their Associations With Harmful Algal Blooms in South Carolina Detention Ponds and Receiving Tidal Creeks

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Abstract Incidences of harmful algal blooms (HABs) and *Vibrio* infections have increased over recent decades. Numerous studies have tried to identify environmental factors driving HABs and pathogenic *Vibrio* populations separately. Few have considered the two simultaneously, though emerging evidence suggests that algal blooms enhance *Vibrio* growth and survival. This study examined various physical, nutrient, and temporal factors associated with incidences of HABs, *V. vulnificus*, and *V. parahaemolyticus* in South Carolina coastal stormwater detention ponds, managed systems where HABs often proliferate, and their receiving tidal creek waters. Five blooms occurred during the study (2008–2009): two during relatively warmer months (an August 2008 cyanobacteria bloom and a November 2008 dinoflagellate bloom) followed by increases in both *Vibrio* species and *V. parahaemolyticus*, respectively, and three during cooler months (December 2008 through February 2009) caused by dinoflagellates and euglenophytes that were not associated with marked changes in *Vibrio* abundances. *Vibrio* concentrations were positively and significantly associated with temperature and dissolved organic matter, dinoflagellate blooms, negatively and significantly associated with suspended solids, but not significantly correlated with chlorophyll or nitrogen. While more research involving longer time series is needed to increase robustness, findings herein suggest that certain HAB species may augment *Vibrio* occurrences during warmer months.

1. Introduction

Incidences of shellfish and water contamination by bacterial pathogens of the genus *Vibrio* and by harmful algal blooms (HABs) caused by a variety of species have increased over several decades (Baker-Austin et al., 2013; Glibert et al., 2005; Hallegraeff, 1993; Heisler et al., 2008; Lewitus et al., 2012; McLean & Sinclair, 2012; Newton et al., 2012; Van Dolah, 2000). The majority of *Vibrio* illnesses arise from seafood consumption, but 12–28% of cases result from direct contact with seawater (Ralston et al., 2011). Two *Vibrio* spp., *V. parahaemolyticus* and *V. vulnificus*, account for 50–60% and 10–15%, respectively, of reported infections in the United States (U.S.) (http://www.cdc.gov/nationalsurveillance/cholera-vibrio-surveillance.htm). *V. parahaemolyticus* is relatively more common and causes gastrointestinal distress. By comparison, *V. vulnificus* causes both gastrointestinal distress and severe dermatitis in healthy individuals, and it is responsible for the majority of *Vibrio* deaths (Daniels, 2011). Mortality occurs through septicemia in immunocompromised individuals with reported rates of 31– >50% and 18–24% for seafood-borne and wound-related infections, respectively (Oliver, 2005, 2013; Ralston et al., 2011; Scallan et al., 2011). Consequently, *V. vulnificus* is the most costly marine pathogen in terms of economic impact (Ralston et al., 2011).

While incidences of HABs have increased globally, coastal zones of the southeastern U.S. are particularly vulnerable to bloom events, due in part to the region having among the most rapid urbanization and population increases in the nation. For example, in South Carolina (SC), rates of land development often exceed human

population growth rates (Allen & Lu, 2003; Holland et al., 2004; Vernberg & Vernberg, 2001). As part of this development, detention ponds are commonly constructed as a best management practice to offset impervious surface development, mitigate stormwater runoff, and reduce coastal flooding (Drescher et al., 2007; Lewitus et al., 2003). Prior studies identified approximately 14,000 detention ponds along the SC coast alone (Smith, 2012); however, construction of additional ponds is ongoing such that a recently conducted inventory revealed that SC coastal detention pond numbers now exceed 21,000 (E. Smith, personal communication). These shallow (<3 m depth), small (area typically 0.4–4 ha or less) systems are infrequently flushed, span a range of salinities (fresh to polyhaline), and are thus associated with high volume residence times making them susceptible to stagnation, particularly during warmer months (late summer through early fall) (Bunker, 2004; Lewitus et al., 2003, 2008; Vandiver & Hernandez, 2009). Since they are often built within residential and recreational (golf course) areas that have extensive landscape maintenance, detention ponds accumulate nutrients (specifically nitrogen, N and phosphorus, P) from fertilizer runoff (DeLorenzo et al., 2012; Drescher et al., 2011; Lewitus et al., 2003). The resultant eutrophication makes them susceptible to persistent, toxic, and recurrent HABs (Lewitus & Holland, 2003; Lewitus et al., 2003, 2008; Drescher et al., 2011; Reed et al., 2016).

Both *V. parahaemolyticus* and *V. vulnificus* have been identified in SC coastal waters (Deeb, 2013; Tufford et al., 2014), and Motes et al. (1998) detected *V. vulnificus* in SC oysters. Thus, it is reasonable to investigate whether similar environmental conditions may favor the proliferation of *Vibrios* and HABs together. Numerous studies have examined the causes and consequences of HABs and *Vibrio* spp. separately, such as for shellfish poisoning (e.g., Daniels, 2011; Lopez et al., 2008; Scallan et al., 2011), but few have considered the two simultaneously. However, *Vibrio cholerae* has been shown to increase in response to bloom filtrate of the "red tide" dinoflagellate *Lingulodinium polyedrum* in microcosms (Mourino-Pérez et al., 2003). *Vibrio* spp. have also been associated with zooplankton, diatoms, and organic particles (e.g., Amin et al., 2012; Takemura et al., 2014; Turner et al., 2014), and abundances have been observed to increase in tandem with a diatom bloom (Gilbert et al., 2011) and correlate with numbers of raphidophytes, diatoms, and dinoflagellates in the Delaware Inland Bays (Main et al., 2015). Therefore, it is reasonable to assess whether HABs may be vectors that augment *Vibrio* bloom formation.

The overall goal of this study was to assess whether phytoplankton blooms, particularly HABs, may be associated with increases in *Vibrio* spp. abundances. Given their widespread use for coastal stormwater management, combined with their tight association with humans and thus likelihood of presenting a public health concern, we focused on SC detention ponds and receiving tidal creek waters to address the following objectives: (1) evaluate the occurrences and concentrations of *V. parahaemolyticus* and *V. vulnificus* in field samples (2008–2009); (2) assess standard water quality, nutrients, and phytoplankton (particularly HABs), specifically as they may relate to *Vibrio* abundances; and (3) generate a model evaluating associations between *Vibrios*, water quality, nutrients, and phytoplankton. This study is one component of a broader study comparing *Vibrio* incidences between two distinct environments (SC and WA) see Paranjpye et al. (2015).

2. Materials and Methods

2.1. Site Descriptions

Kiawah Island (KI) is a barrier island ~45 km southwest of Charleston, SC. It is characterized by extensive landscaping, golf courses, and networks of detention ponds as catchments for stormwater runoff (Lewitus et al., 2003, 2008; Holland et al., 2004). There are 116 detention ponds on KI within ~335 acres (1.356 km^2), the majority of which are shallow (1–3 m depth), brackish to marine systems (Lewitus et al., 2003). Monitoring and research of water quality, nutrients, and phytoplankton in KI detention ponds have been ongoing since 2001, and these efforts have shown them to be "hot spots" for HAB development. Recurrent KI blooms span a wide range of phytoplankton taxa including the cyanobacteria genera *Microcystis*, *Anabaenopsis*, *Oscillatoria*, *Cylindrospermopsis*, and *Aphanizomenon* (Brock, 2006; Greenfield et al., 2014; Lewitus et al., 2008; Siegel et al., 2011), the raphidophyte species *Heterosigma akashiwo*, *Fibrocapsa japonica*, *Chattonella* spp., and *Viridilobus marinus* (Lewitus et al., 2003, 2008; Keppler et al., 2006; Reed et al., 2016), the dinoflagellates *Scrippsiella* spp., *Prorocentrum minimum*, and *Karlodinium veneficum*, (Lewitus et al., 2003, 2008), and the haptophyte *Prymnesium parvum* (Lewitus & Holland, 2003; Lewitus et al., 2003, 2008).

Sites used in this study, KI pond numbers K001 (K001 Pond henceforth and similar notation throughout) and K075 Pond, are shallow (<3 m depth), polyhaline ponds that connect directly to receiving tidal creek

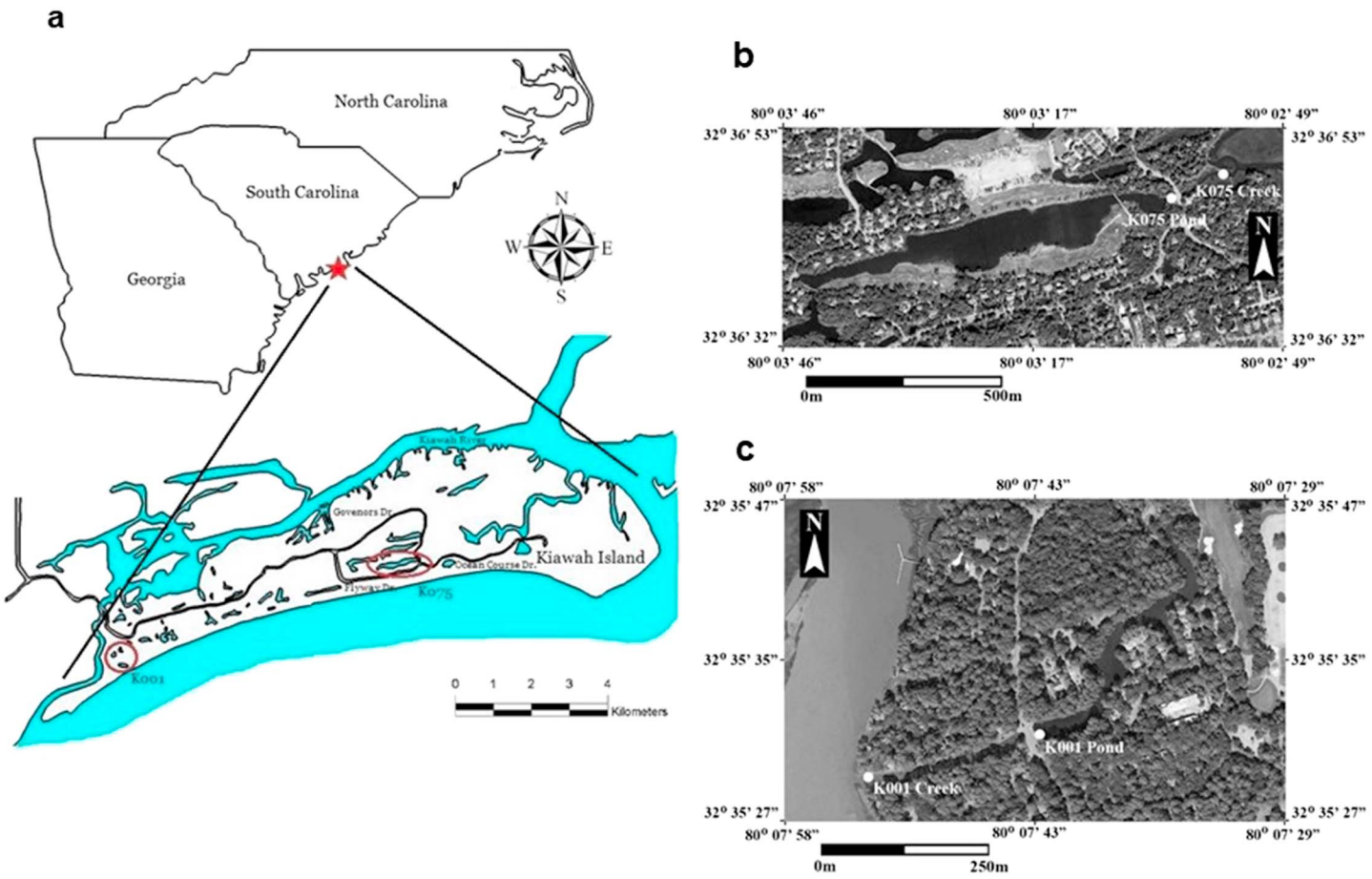

Figure 1. Map depicting study location within South Carolina (SC) USA as (a) a regional overview of study area along the southeastern coast, highlighting Kiawah Island (star and insert), as well as the (b) K075 and (c) K001 systems, with sampling locations indicated (white circles) for both ponds and receiving tidal creeks.

estuaries draining to the Kiawah River (Figure 1), such that the K075 Pond sampling location is 7.11 km west of the K001 Pond sampling location. K001 Pond has an area of 1.4 ha, an average depth of 1.5 m, contains 2.10×10^4 m^3 of water (N. Shea, personal communication), and has an annual average salinity of 21 psu. It is the terminus of a system of connected ponds that receives water from the western half of KI such that four stormwater pipes drain to K001 Pond and four different pipes connect it with a tidal creek. Surrounding land use includes road, residential housing, and a buffer zone (<10 m) of wax myrtle trees. K075 is larger than K001 with an area of 9 ha, an average depth of 1.7 m, contains 1.55×10^5 m^3 of water (N. Shea, personal communication), and has an annual average salinity of 13 psu. Like K001 Pond, K075 Pond is the final in a system of connected ponds, but it drains water from the southeastern portion of KI such that 15 stormwater pipes drain to K075, and four other pipes connect it to a tidal creek. Surrounding land use includes road, residential housing, a golf course, and wax myrtles (Reed et al., 2016).

2.2. Sample Collection

K001 and K075 Ponds, as well as sites ~200–225 m downstream within receiving tidal creeks (henceforth K001 Creek and K075 Creek, respectively) located by the confluence of an adjacent estuary (Figure 1), were sampled twice per month during mid-ebb (Kiawah tidal cycles are semidiurnal) 2 April 2008 to 18 August 2009. Standard water quality parameters (temperature (°C), salinity (psu), and dissolved oxygen (DO, mg L^{-1})) were recorded from surface (0.3 m) depths using a hand-held YSI 85 unit, and pH was measured using a hand-held pH meter. Triplicate 1 L water samples were collected using opaque Nalgene bottles that were previously acid-washed (immersion in 10% hydrochloric acid, HCl, for at least 4 h followed by rinsing 3 times with deionized water), placed in the dark inside a cooler, then immediately transported to the laboratory for processing.

2.3. Chlorophyll Processing and Analyses

Upon return to the laboratory, whole water samples (up to 40 mL) from each replicate were filtered through a Whatman 0.7 μm glass fiber filter (GF/F) for total chlorophyll *a* (Chl *a*) (a commonly used proxy for phytoplankton biomass) following standard methods for nonacidification of the pigment (Arar & Collins, 1997). Briefly, filters containing sample were placed into HCl-washed (as above) 25 mL scintillation vials, 1 mL of saturated magnesium carbonate ($MgCO_3$) was added as a buffer to prevent acid degradation of pigment, and samples were frozen (−20°C) until analysis. To evaluate Chl *a* levels, 9 mL of high-performance liquid chromatography (HPLC) grade acetone (90%) was added to each replicate, pigment was extracted (−20°C for 36 h), then Chl *a* concentrations (μg L^{-1}) were evaluated using a Turner Design 700 fluorometer.

2.4. Nutrient Processing and Analyses

Aliquots (25 mL) of whole water from each sample replicate were either dispensed directly into acid-washed (as above) scintillation vials for total nitrogen (TN) and phosphorus (TP) or filtered through pre-combusted (450°C for 4 h) GF/F filters, and filtrate was collected in scintillation vials for orthophosphate (PO4), nitrite + nitrate combined (N+N), ammonium (NH4+), silicate (Si), and total dissolved nitrogen and phosphorus (TDN and TDP, respectively). Subsequent evaluation of nutrient concentrations (μM) used a Lachat QuickChem 8000 nutrient auto-analyzer with an ASX 500 autosampler, following well-established methods (Grasshoff et al., 1999; Johnson & Petty, 1983; Zimmerman & Keefe, 1991). Dissolved organic nitrogen (DON) and phosphorus (DOP) were calculated by subtracting the dissolved inorganic nutrients from TDN and TDP, respectively. Dissolved organic carbon (DOC) was sampled as above followed by acidification with 1 drop of 10% HCl, then stored (4°C) until concentrations (μM) were determined using a Shimadzu TOC-V CSN analyzer with ASI-V autosampler according to manufacturer specifications. Prior to each sampling batch (30–60 samples), a SIX-point calibration curve was generated according to standard protocols (Shimadzu TOC-$V_{CSH/CSN}$ User Manual). Concentrations (mg L^{-1}) of total suspended and volatile suspended solids (TSS and VSS, respectively) were determined by condensing ~200 mL of whole water on to preweighed and precombusted (as above) 47 mm GF/F filters, placing filters in a drying oven for 3 days, weighing (TSS), combusting again, then reweighing (VSS).

2.5. Phytoplankton Community Analyses

Qualitative phytoplankton community composition was determined by sampling an aliquot (~2–3 mL) of whole water from one replicate (chosen at random), dispending into a Lab-Tek II 2-chamber slide, settling for 5 min, and then viewing (40X magnification) with a Nikon Eclipse TS100 inverted microscope. All observed phytoplankton were visually identified to the lowest taxonomic level (LTL) possible. Bloom species (bloom concentrations vary widely among taxa) were quantified (cells mL^{-1}) by preserving an aliquot (100 mL) from the same replicate with Lugol's iodine solution (3% final preservative concentration), then individual cells were enumerated using a 0.1 mm or 0.5 mm haemocytometer until either a minimum of 300 cells or the entire chamber was counted, whichever occurred first.

2.6. Evaluation of Vibrio Spp.

To evaluate the presence and abundances of *V. parahaemolyticus* and *V. vulnificus*, each replicate sample (up to 100 mL) was filtered through a 0.45 μm, 47 mm, polyethersulfone (PES) membrane filter (Sterlitech Corporation, Kent, WA) on a sterile vacuum manifold until the filter clogged. The volume of filtered water was recorded, then each filter was placed in a sterile, 50 mm petri dish with a tight-fitting lid (VWR) and frozen (−80°C) until analysis. DNA was extracted following Boström et al. (2004), modified to increase the volumes of lysis buffer to 2 mL and the lysozyme to 105 μL before incubating (30 min at 37°C) on an orbital shaker. Remaining cell lysis and DNA recovery followed Boström et al. (2004). DNA was treated with the PowerClean DNA Clean-Up kit (Mobio Laboratories, Carlsbad, CA) to remove PCR inhibitors. Inhibition was assessed by examining the sample C_T values, and any that exceeded 2 standard deviations above the standard C_T value were considered inhibited and diluted twofold.

Two variables for total *Vibrio* spp. densities (genome equivalent units (GEUs) per 100 mL, henceforth GEU 100 mL^{-1}) were included: *V. parahaemolyticus tlh* gene and *V. vulnificus vvhA* gene. In addition, potential *V. parahaemolyticus* virulence markers (*tdh* and *trh* genes) were evaluated using a TaqMan based real-time multiplex PCR assay (Nordstrom et al., 2007). *V. vulnificus* detection followed the real-time PCR method of Panicker and Bej (2005) using genomic DNA and the primers F-vvh785 and R-vvh990 along with P-vvh875, which target

the *vvhA hemolysin A* gene. The source strains were ATCC *V. parahaemolyticus* 17802 (genes *tdh*, *trh*, and *tdh*) and *V. vulnificus* 27562 (gene *vvhA*--hemolysin A gene). Each triplicate water sample was analyzed 3 times for a total of nine data points using a BioRad iQ5 and BioRad's Mastermix. For all *Vibrio* spp. variables, the limit of detection (LOD) was ~11 copies 100 mL^{-1} based on standard curves. A positive control *Vibrio* strain and a negative extraction control (containing no *Vibrio*) were included with each PCR run.

2.7. Modeling and Statistical Analyses

Descriptive and statistical analyses were performed using SAS 9.3 (SAS Institute Inc., 2011, Cary, NC, USA). Since preliminary results revealed that the *tdh* and *trh* genes were not detected in any sample, two variables for total densities of *Vibrio* spp. were included: *V. parahaemolyticus* (GEU 100 mL^{-1} of the *tlh* gene), and *V. vulnificus* (GEU 100 mL^{-1} of the *vvh* gene). Since both genes had one copy per genome, GEU values were converted to copies per 100 mL (henceforth, copies 100 mL^{-1}). Phytoplankton species were grouped into chlorophytes, cyanobacteria, euglenophytes, diatoms, raphidophytes, dinoflagellates, or "other flagellates" (cryptophytes, silicoflagellates, prymnesiophytes, etc.). Each group was categorized as one of four categories: absent, present, abundant, or bloom. Categorical variables were coded as absent (0), present (>0–100 cells mL^{-1}), abundant (>100–10,000 cells mL^{-1}), or bloom (>10,000 cells mL^{-1}).

Five continuous variables for water quality characteristics were evaluated: temperature, salinity, Chl *a*, DO, and pH. Means of 13 continuous nutrient variables were also evaluated: DOC, NH4+, N+N, TN, PO4, Si, TDN, DON, DOP, TP, TDP, TSS, and VSS. Spearman's coefficients of correlation were calculated for pairwise tests of rank order relationships between variables (Table S1 in the supporting information). Logistic regression models were used to evaluate associations of binary response variables (presence/absence) and explanatory variables adjusting for all variables in the models. Since *Vibrio* spp. abundances were below the detection limit on numerous sampling dates, we modeled the presence or absence of *Vibrio* spp. as an outcome using logistic regression with explanatory water quality characteristics, nutrients, and phytoplankton. Models were fit for location and years separately and combined, using stepwise selection with an entry significance level set at 0.7 and a stay significance level set at 0.1. The location reference group was K001 Creek. The stepwise selection was initiated on full models composed of the categorical phytoplankton, the five continuous water quality characteristics, and selected continuous nutrients. The lowest phytoplankton category was used as the reference in each model. Nutrients included in the full models were DOC, NH4+, N+N, PO4, Si, DON, DOP, TDP, TSS, and calculated variables for particulate nitrogen (PN) and particulate phosphorous (PP) as PN = TN − TDN and PP = TDP − TDP, respectively. Certain metrics (TN, TDN, TP, and VSS) were omitted from the full models, because they were not independent of the included variables. The models resulting from the stepwise selection were refit using Firth's penalized likelihood estimation for bias reduction in the parameter estimates, which was applied where model convergence was affected by sparse categorical data (Firth, 1993; Heinze, 1999). Collinearity diagnostics based on Belsley et al. (1980) were examined for water quality characteristics and nutrients in the final models using the SAS REG linear regression procedure that omitted categorical variables. The diagnostics found no collinearities of concern, consistent with stepwise selection in the SAS LOGISTIC procedure that removed variables not highly correlated with *Vibrio* spp. presence.

3. Results

3.1. Distribution and Occurrences of *Vibrio* Spp.

A total of 136 water samples were analyzed for *V. parahaemolyticus* and *V. vulnificus* over the study period. *V. parahaemolyticus* was detected in 53 (39%) of these samples whereas *V. vulnificus* was detected in 38 (28%) samples. Concentrations of both *Vibrio* species were greater during 2009 than 2008 such that maximum values (2009) were *V. parahaemolyticus* (3.18×10^4 copies 100 mL^{-1} on 6 July) and *V. vulnificus* (1.07×10^4 copies 100 mL^{-1} on 3 August), both in K075 Creek (Figure 2).

More samples tested positive for *Vibrio* in the K075 system than the K001 system (Figure 2), but there was no clear pattern in *Vibrio* occurrences between ponds and their associated tidal creeks. During 2008, *V. parahaemolyticus* was detected (abundances > LOD at least 1X/month) in the K001 system June–November except July and in the K075 system May–November except June and August (Figure 2a). During 2009, *V. parahaemolyticus* was detected in the K001 system January–August except April and in the K075 system January–August except February. By comparison, *V. vulnificus* was detected in both systems during 2008 April and

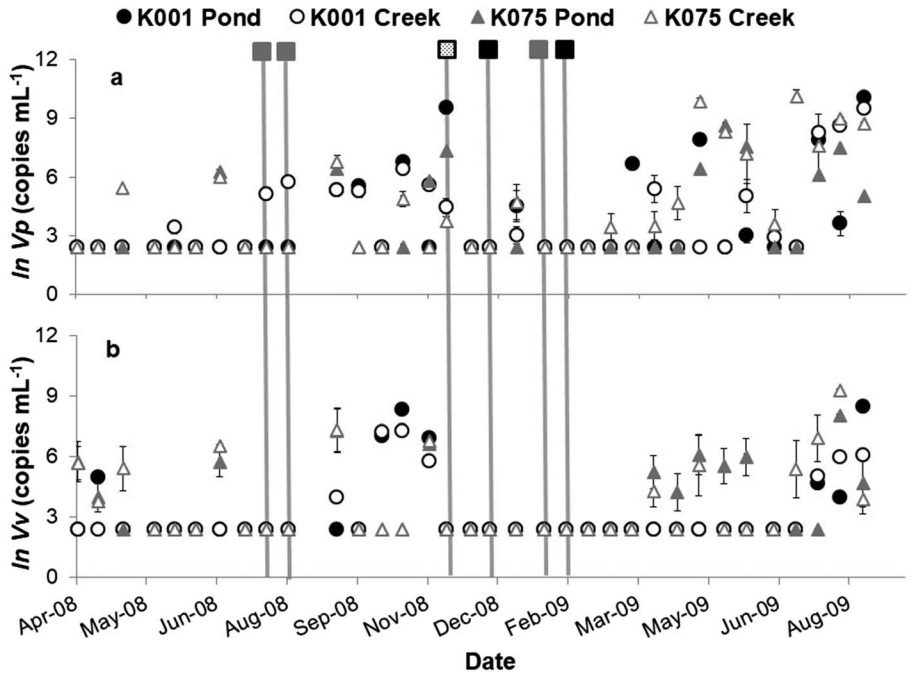

Figure 2. Time series of *Vibrio* spp. concentrations (mean *ln* copies 100 mL^{-1} ± SE) at the four study locations as (a) *V. parahaemolyticus* (V_p), and (b) *V. vulnificus* (V_v). Concentrations that were nondetect are set to the limit of detection (*ln* (11) = 2.4). Black circles denote K001, grey triangles denote K075, with filled and open symbols representing pond and creek data, respectively. Squares indicate dates when a HAB was noted in a field sample such that black denotes the K001 system, grey denotes the K075 system, and speckled denotes a HAB observed in both systems, connected through each system by the solid grey line. Causative bloom organisms were *Cylindrospermopsis raciborskii* (1 August 2008), *Anabaena* sp. (15 August 2008), *Heterocapsa rotundata* (24 November 2008), *Karlodinium veneficum* (26 January 2009), and *Eutreptiella* sp. (22 December 2008, 9 February 2009).

September–November, as well as K075 Creek May. During 2009, it was only detected in the K001 system July and August compared to the K075 system April–August (Figure 2b).

3.2. Association Between *Vibrio* spp. and Phytoplankton Community Composition

Observed phytoplankton spanned 137 categories (identified to the LTL possible). The most frequently observed taxa (cells >2 μm diameter) were dinoflagellates, diatoms, and raphidophytes, which were present in 98%, 85%, and 43% of water samples, respectively (Table 1). However, "other flagellates" were present in 74% of all samples.

Five algal blooms were identified during the study (Figure 2), and they represented three taxonomic groups: cyanobacteria, dinoflagellates, and euglenophytes. HAB events included a cyanobacteria bloom, initially dominated by *Cylindrospermopsis raciborskii* (1.20×10^4 cells mL^{-1}) then *Anabaena* (1.90×10^4 cells mL^{-1}) on 1 and 15 August 2008, respectively, in the K075 system. This multispecific event was followed by increases from nondetect of both *Vibrio* spp. to 6.82×10^2 and 1.17×10^3 copies 100 mL^{-1} of *V. parahaemolyticus* and 5.10 and 5.16×10^3 copies 100 mL^{-1} of *V. vulnificus* in K075 Pond and Creek, respectively, on 15

Table 1
Percent Observations (n = 34 Samples per Site, N = 136 Total Sampling Events) of Each of the Seven Major Phytoplankton Taxonomic Groups Considered Herein (Taxon was Present in Samples), According To Study Site and Averall

	Phytoplankton group						
Site	Dinoflagellates	Diatoms	Raphidophytes	Euglenophytes	Other Flagellates	Chlorophytes	Cyanobacteria
K001 Pond	97	82	56	47	76	24	12
K001 Creek	100	100	26	24	71	21	0
K075 Pond	100	71	47	29	79	32	21
K075 Creek	97	88	41	32	68	44	18
Total	98	85	43	33	74	30	13

Table 2
Logistic Regression for Categorical Phytoplankton Fit for Combined Years, Using Stepwise Selection

		Overall	
	Variable	Estimate	*p* value
V_p	Intercept	−1.70	0.04
	K001 Pond	−1.10	0.06
	K075 Creek	−0.75	0.20
	K075 Pond	−1.91	<0.001
	Dinoflagellates abundant	−0.85	0.50
	Dinoflagellates bloom	2.78	0.03
	T	0.16	<0.001
	TSS	−0.02	<0.001
V_v	Intercept	1.09	0.25
	DO	−0.18	0.15
	DOP	−0.12	<0.001
	TSS	−0.02	0.01

Note. V_p Indicates *V. parahaemolyticus* and V_v indicates *V. vulnificus*.

September (Figure 2). Multiple HABs were caused by dinoflagellates during the study period, and dinoflagellate blooms were positively and significantly associated with *V. parahaemolyticus* presence using combined data from both study years (Table 2). For example, dense blooms of the nuisance species *Heterocapsa rotundata* were observed on 24 November 2008 at K001 Pond (9.92×10^4 cells mL^{-1}), K075 Pond (3.82×10^4 cells mL^{-1}), and K075 Creek (1.15×10^4 cells mL^{-1}). Each *H. rotundata* bloom coincided with increased *V. parahaemolyticus* numbers relative to 13 November as K001 Pond (nondetect to 2.00×10^4 copies 100 mL^{-1}), K075 Pond (3.41×10^3 to 1.41×10^4 copies 100 mL^{-1}), and K075 Creek (nondetect to 62.9 copies 100 mL^{-1}) (Figure 2 a). However, *V. vulnificus* was nondetect on 24 November at all sites (Figure 2b). By comparison, a bloom of *Karlodinium veneficum* (3.15×10^4 cells mL^{-1}) that occurred at K075 Creek on 26 January 2009 was not associated with increased *Vibrio* spp. abundances. Finally, blooms of the non-HAB euglenophyte *Eutreptiella* sp. occurred at K001 Pond on 22 December 2008 (2.65×10^4 cells mL^{-1}) and 9 February 2009 (4.84×10^4 cells mL^{-1}). The 22 December bloom was followed by small increases in *V. parahaemolyticus* numbers in both K001 Pond (nondetect to 3.90×10^2 copies 100 mL^{-1}) and K001 Creek (nondetect to 5.89×10^1 copies 100 mL^{-1}), but as with *K. veneficum*, neither of these midwinter (January–February) blooms were associated with notable changes in *Vibrio* spp. abundances.

3.3. Associations Between *Vibrio* Spp. and Water Quality

For sites and years combined, Spearman correlations showed that *V. parahaemolyticus* and *V. vulnificus* were significantly ($\alpha = 0.05$) correlated with several water quality and nutrient parameters (Table S1). Specifically, both *V. parahaemolyticus* and *V. vulnificus* positively correlated with each other, Si, TDN, DON, TP, and TDP. *V. parahaemolyticus* negatively and significantly correlated with salinity, whereas *V. vulnificus* positively correlated with temperature but negatively and significantly correlated with DO. At $\alpha = 0.10$, both *Vibrio* spp. positively correlated with TN and *V. parahaemolyticus* positively correlated with pH and DOC, whereas *V. vulnificus* positively correlated with PO4 but negatively correlated with salinity. *Vibrio* spp. abundances were not significantly correlated with Chl *a*, suspended solids, or inorganic N concentrations.

Of the numerous water quality parameters measured during this study, those most closely associated with and relevant to *Vibrio* spp. population changes (versus those that primarily drive phytoplankton dynamics, such as N, P, and Si) are highlighted (Figure 3), though correlations with every measured water quality parameter are also provided (Table S1). On any given sampling date, water temperatures were similar across sites, ranging 7.1–32.0°C. *Vibrio* spp. were generally more abundant during the warmer months, reaching maximum abundances during summer of 2009 coincident with water temperatures of 29.5–31.5°C (Figures 2 and 3a). However, maximum *Vibrio* spp. abundances during 2008 followed November bloom events (described above), coincident with relatively cooler temperatures (10.3–11.6°C; Figure 3a). Salinity was generally higher in the K001 system (mean of 26.7 psu, ranging 9.7–35.0) than the K075 system (mean of 14.4 psu, ranging 4.3–28.0; Figure 3b), with tidal creeks typically more saline than their corresponding ponds. Mean DOC levels averaged 1,272 μM and were usually <2,000 μM but exhibited wide ranges (119–6,766 μM; Figure 3c), due to elevated (5,232–6,766 μM) DOC at K075 Creek April–July 2008. Moderate increases in DOC during 2009 were coincident with increases in both measured *Vibrio* spp. (Figures 2 and 3c), and for *V. vulnificus* this association was highly significant ($p < 0.001$; Table 3). TSS was elevated April–July 2008 at all sites (Figure 3d) as well as during the spring and summer of 2009, coincident with increases in *Vibrio*. During both years, decreases in mean TSS levels were followed by increases in *Vibrio* populations (Figure 3d and Tables 2 and S1).

Spearman's rank correlation coefficients assess two variables at a time without adjustment for other variables and are not directly comparable to the logistic regression models we used for inference. Results from logistic models suggest that overall, dinoflagellate blooms and increased temperature in SC detention pond systems were associated with increased numbers of *V. parahaemolyticus* but not *V. vulnificus*. Due to variability in

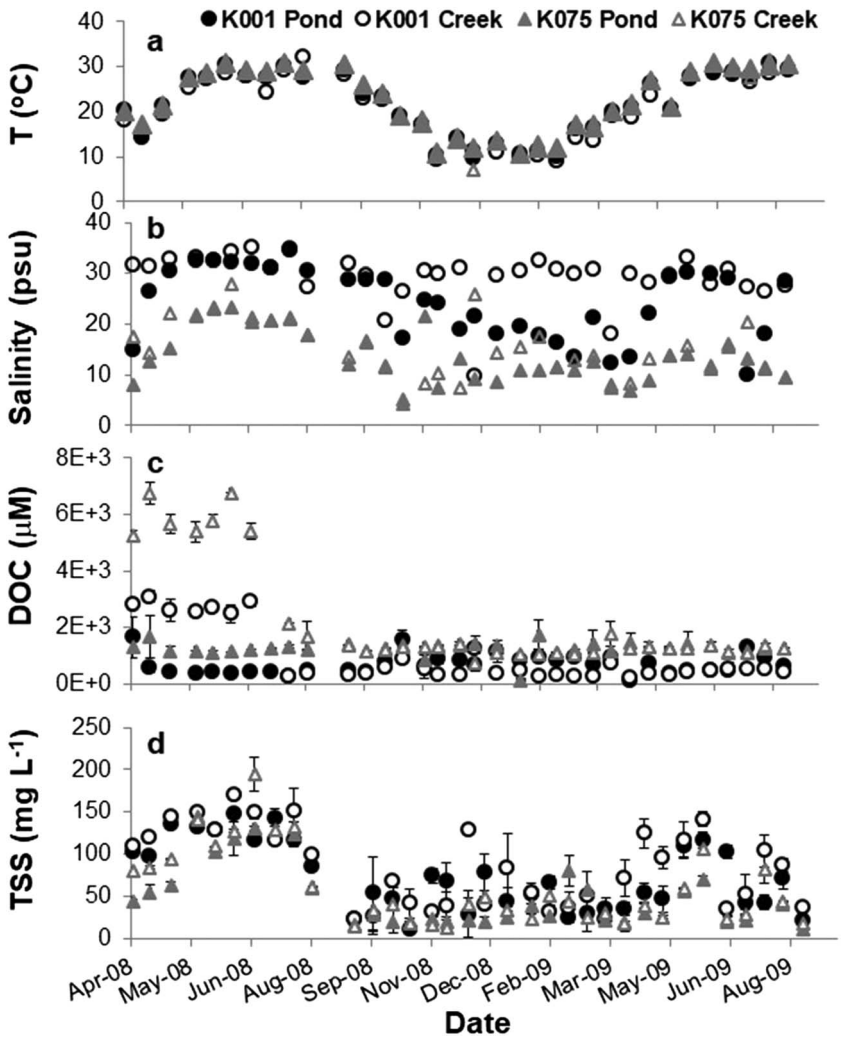

Figure 3. Time series of select water quality parameters at the four study locations used for this study. Metrics include (a) water temperature (T), (b) salinity, (c) dissolved organic carbon (DOC), and (d) total suspended solids (TSS). DOC and TSS values represent mean concentrations ($n = 3 \pm SD$). Black circles denote K001, grey triangles denote K075, with filled and open symbols representing pond and creek data, respectively.

explanatory models among years and locations for both *Vibrio* spp., annual and combined models are provided (Tables 2 and 3). Both *Vibrios* were significantly and negatively associated with TSS, and *V. vulnificus* was significantly and negatively associated with DOP. DO was retained in the model even though it was not significantly associated with *Vibrio* because its inclusion improved the overall fit as determined by Akaike information criteria (AIC), which dropped from 95.13 to 90.44. *V. vulnificus* was significantly and negatively associated with DOP at K075 Pond and positively associated with temperature at K075 Creek (Table 3). Overall, *V. parahaemolyticus* was significantly and negatively associated with K075 Pond and TSS (Table 3), and significantly and positively associated with temperature and with dinoflagellate blooms (Table 2), suggesting that these blooms may act as a vector for *V. parahaemolyticus* persistence, given warmer water temperatures.

4. Discussion and Conclusions

Findings herein suggest that HABs in SC detention pond systems were associated with increased numbers of both *Vibrio parahaemolyticus* and *V. vulnificus*, but only when waters were relatively warm (>10°C). *Vibrio* spp. abundances increased following the August and November HABs caused by cyanobacteria and

Table 3
Logistic Regression for Categorical Phytoplankton Fit for Individual Years, Using Stepwise Selection

			Overall		K001 Pond		K075 Creek		K075 Pond	
		Variable	Estimate	*p* value	Estimate	*p* value	Estimate	*p* value	Estimate	*p* value
2008	V_p	Intercept	−4.24	0.03	1.76	0.22			−2.04	0.02
		Salinity	0.20	0.01						
		PO4	0.07	0.06						
		TSS	−0.03	<0.001	−0.04	0.05				
		Euglenophytes							2.04	0.09
	V_v	Intercept			−3.52	0.03			−1.61	0.02
		PO4			0.19	0.09				
		Chlorophytes							2.46	0.06
2009	V_p	Intercept	−6.50	<0.001			−4.67	0.09	−0.36	0.55
		T	0.21	<0.001			0.30	0.05		
		DOC	0.00	0.09						
		N+N	0.93	0.04						
		DOP							−0.07	0.07
	V_v	Intercept	0.32	0.80	−5.09	0.03	−5.19	0.05	−0.03	0.96
		DO	−0.82	<0.001						
		DOC	0.00	<0.001						
		PO4			0.19	0.06				
		T					0.20	0.05		
		DOP							−0.07	0.05

Note. V_p Indicates *V. parahaemolyticus* and V_v indicates *V. vulnificus*. Metrics include temperature (*T*), total suspended solids (TSS), dissolved oxygen (DO), dissolved organic phosphorus (DOP), dissolved organic carbon (DOC), and nitrate + nitrite (N + N) and the presence of euglenophytes and chlorophytes.

dinoflagellates, respectively, but *Vibrio* spp. numbers were not augmented by winter dinoflagellate and euglenophyte blooms, coincident with relatively colder water temperatures. These observations support Turner et al. (2009) who found that seasonality affected *Vibrio* spp. abundances and their associations with plankton. In our study, the positive and significant association between temperature and *V. parahaemolyticus* during 2009, combined with the positive and significant correlation between temperature and *V. vulnificus*, confirms that warmer conditions favor *Vibrio* spp. population numbers in SC coastal detention pond systems. Positive correlations between both *Vibrio* spp. and water temperature have been observed elsewhere, though substantial variation exists among studies and geographies (Johnson, 2015; Ramirez et al., 2009; Takemura et al., 2014; Turner et al., 2014). Nevertheless, water temperature is generally considered an important predictor of *V. parahaemolyticus* and *V. vulnificus* occurrences (e.g., Baker-Austin et al., 2013; Grimes et al., 2014).

Although Spearman correlations revealed negative and significant correlations between both *Vibrio* spp. and salinity, our model did not predict an overall significant effect of salinity on *V. parahaemolyticus* when years were combined. These findings contrast prior research showing positive correlations between salinity and *Vibrio* spp. incidences (e.g., Baker-Austin et al., 2013; Johnson, 2015; Tufford et al., 2014). Since *V. parahaemolyticus* and salinity were positively and significantly correlated during 2008 when salinities were highest, interannual variability may be a factor. Another explanation could be that the majority of previous studies entailed relatively more open (oceanic) waters with short residence times in sharp contrast to the long residence times that characterize residential detention ponds.

Our model showed that *Vibrio* spp. were negatively associated and during 2008 *V. parahaemolyticus* was significantly associated with TSS. The cause(s) of elevated TSS and DOC during spring of 2008 are unclear; heavy storms are unlikely because state climate records indicate this was a period of drought (http://www.dnr.sc.gov/water/climate/sco/Drought/drought_current_info.php). It is possible that golf course irrigation washed material into the waters, but management records are proprietary and consequently unavailable for this study. However, since neither *Vibrio* species was significantly related with Chl *a*, this study suggests that particulate load does not always translate to available *Vibrio* attachment sites. Moreover, *Vibrio* may not require their substrates to be phytoplankton. Since our model predicted a significant association between *V. parahaemolyticus* and certain taxa (dinoflagellates, chlorophytes, and euglenophytes), as well as a

positive correlation with DOC, particle type rather than overall load may be important drivers of *Vibrio* population numbers in the systems considered here. Certain phytoplankton taxa tend to have "leaky" cell membranes and generate relatively higher levels of DOC. Dinoflagellates and raphidophytes, as examples, produce high levels of cellular exudates (Seymour et al., 2009; Takemura et al., 2014) thus contributing proportionally higher to the DOC pool. In fact, nutrient incubation studies conducted in K075 Pond showed that dinoflagellate HABs are not only stimulated by DON (as urea) additions (Reed et al., 2016), but these blooms are also followed by increased DOC levels (Reed et al., 2015). Therefore, blooms of dinoflagellates and potentially other taxa could produce high levels of DOC that may, in turn, facilitate *Vibrio* spp. abundances and/or provide particulates with multiple attachment sites (Frischkorn et al., 2013; Mourino-Pérez et al., 2003).

This study, part of a broader assessment of how HAB incidences may relate to *Vibrio* occurrences in SC and Puget Sound, WA (Paranjpye et al., 2015), underscored how variability between sites may drive microbial populations. Examples of differences between the two studies included the following: (1) the potentially virulent *tdh*+ strain of *V. parahaemolyticus* was abundant in WA, whereas this gene was not detected in SC samples; (2) *V. vulnificus* was absent from WA samples; (3) opposite correlations with Si (negative in WA versus positive in SC) for *V. parahaemolyticus*, which is notable because the HAB diatom *Pseudo-nitzschia* spp. is commonly found along coastal WA (e.g., Hubbard et al., 2014; Trainer et al., 2009); and (4) Paranjpye et al. (2015) did not find correlations between *V. parahaemolyticus* and HABs or other phytoplankton, though no blooms were detected during their study. However, neither study showed significant correlations between *Vibrio* spp. abundances and Chl *a* levels, underscoring that phytoplankton per se are not required for *Vibrio* to proliferate. Both studies indicate that *Vibrio* are likely opportunistic and use multiple substrates (dissolved and particulate), including phytoplankton when they become highly abundant (such as during a bloom), supporting previous research (Gilbert et al., 2011). Since HABs can be dense and highly persistent, blooms may provide convenient attachment sites and thus favor increases of *Vibrio* populations to potentially dangerous levels.

Work presented here has broader public health and environmental implications as future climate scenarios suggest that summers will become longer and warmer in the mid-Atlantic and Southern states (Cronin et al., 2003; Najjar et al., 2010; National Climate Assessment, 2014). These conditions are likely to lead to prolonged periods of stagnation (e.g., Michalak et al., 2013; Paerl & Huisman, 2009; Paerl & Paul, 2012) which may facilitate the development and proliferation of HABs (Paerl & Huisman, 2009; Paerl & Paul, 2012), particularly in shallow detention ponds such as those considered here. Rising temperatures could also drive *Vibrio* population increases (Baker-Austin et al., 2013). Combined, these predictions suggest future scenarios of increased HABs and *Vibrio*. Results from this study suggest that certain HABs, particularly dinoflagellates and potentially other taxa, could provide an environment conducive to *Vibrio* growth, perhaps augmented by the release of cellular exudates. Additional studies should focus on specific phytoplankton taxa and their tendency to be associated with *Vibrio* as well as continue to explore the extent to which organic matter facilitates *Vibrio* population numbers. Finally, few other studies have examined the occurrences of pathogenic *Vibrios* in saline U.S. ponds. Cox and Gomez-Chiarri (2012) reported *V. parahaemolyticus* from two saltwater ponds in Rhode Island, and DeLorenzo et al. (2012) reported occurrence of *V. parahaemolyticus* and *V. vulnificus* throughout the year in water in a receiving creek immediately adjacent to a residential detention pond in Charleston, SC. Their studies and our specific findings of *V. parhaemolyticus* and *V. vulnificus* in SC, both detention ponds and receiving creeks, underscore the potential for these systems to harbor a direct health risk to people who reside near them and/or their use them for recreation. Considering the continued widespread use of detention ponds in many residential communities throughout the Southeastern U.S., this is an issue that should be the focus of considerable future research, especially in light of projected climate change effects.

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